Hagfish protein threads as three dimensional in vitro scaffolds for skeletal muscular tissue

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Introduction

Traditional *in vitro* models of skeletal muscular tissue:

- Are typically grown in monolayer or suspended culture (Fig 1)
- Are not accurate representation of *in vitro* conditions

A more accurate model skeletal muscle tissue:

- Can be made using protein threads synthesized from hagfish slime
  - Suspended on acrylic chassis (Fig 2).
  - Increased throughput
- More accurately represent muscle fiber as occur naturally *in vivo*

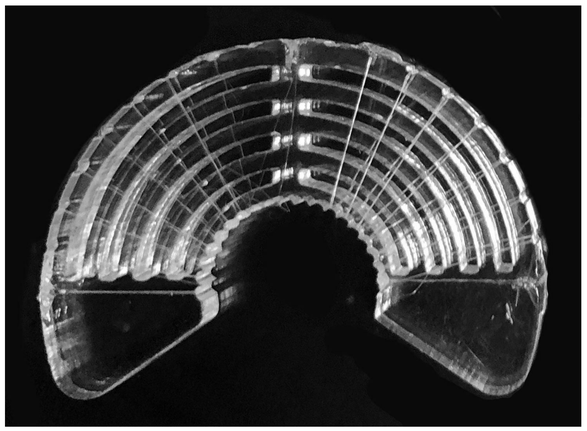


Figure 2 - 6-fiber bundles of hagfish protein thread wound around an acrylic chassis

Methods

To create this model, the following steps were performed:

1. Threads were synthesized from recombinant hagfish proteins harvested from genetically modified *E. coli*
2. Threads were combined into bundles of 6 and wound around an acrylic chassis, sterilized using ultraviolet light, and plasma-treated
3. The wound chassis were seeded with C2C12 mouse myoblasts
4. The growth of the C2C12 culture was evaluated using brightfield microscopy for 7 days after differentiation

Results

These cultures showed:

- Growth along the hagfish protein thread (Fig 3)
- Independent attachment to acrylic chassis (Fig 3)

Conclusions

These results show that hagfish thread suspended using an acrylic chassis can:

- Support C2C12 cell growth
- Form three dimensional cultures along the hagfish protein thread without additional support
- Facilitate independent cell anchorage to acrylic, mimicking the attachment of tendon to bone
- More accurately model *in vivo* skeletal muscle tissue than monolayer and suspension cultures

Future Work

- Fluorescent and immunocytochemical staining to analyze cell viability, cell morphology, and myofiber alignment.

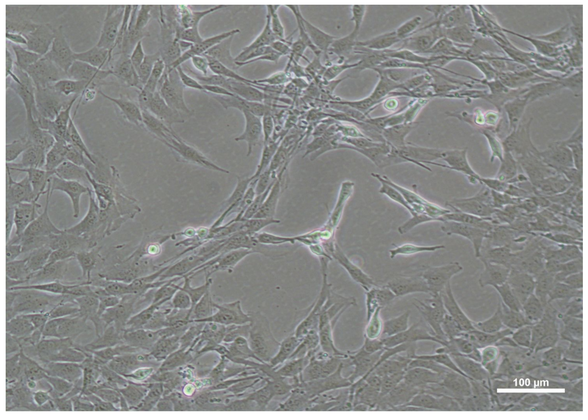
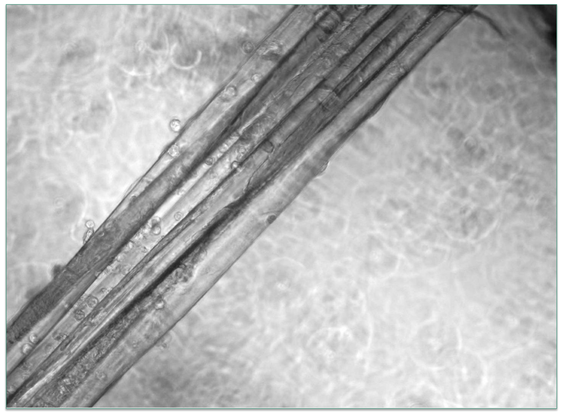


Figure 1 - C2C12 culture growing as a monolayer



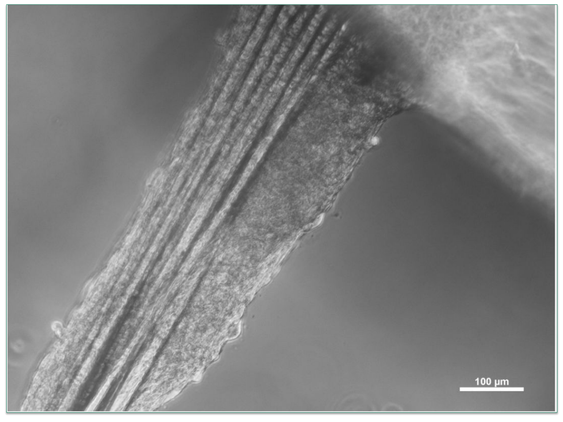


Figure 3 - 3D C2C12 culture at 1 days post seeding (left) and 3 days post seeding (right)